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| **Modified Giemsa for Malaria Stain** |
| **Purpose** | To aid in identification of all blood parasites. |
| **Policy Statements** | This procedure applies to Histology Technical staff performing special stains. |
| **Principle** | During some stages in their life cycle, species of Plasmodium, Babesia, Trypanosoma, Leishmania donovani, and filaria are detectable in human blood. Plasmodium and Babesia species are found within the RBCs; trypanosomes and microfilariae, the larval stage of filariae, are found outside the RBCs; and Leishmania amastigotes are occasionally found within monocytes. Trypanosomes and microfilariae, which frequently are present in low numbers, exhibit motility in freshly collected blood smears, and this can aid in their detection. |
| **Materials** | **Supplies** | **Reagents** |
|  | • PPE • Graduated cylinders• Disposable pipets• Coplin jars with lids | • Phosphate Buffer Solution, pH 7.0  • Triton X   |
| **Sample** | Four thin peripheral blood smears (routine) and four thick blood preparations are prepared by Hematology. The thick preparation slides are made by placing one drop of blood on a glass slide and then spreading the blood over the slide in a circle to the size of a nickel, then air drying well. |
| **Quality Control** | Use a smear with known organisms, usually purchased in a fixed state. \*Note: Occasionally, there are patient blood smears that are strongly positive for Malaria organisms. Control smears may be made, dated and used from these samples ONLY with prior Pathologist approval. These controls may be used unfixed or fixed, as the purchased control slides. Unfixed blood smears for Malaria controls should be stored at room temperature, shielded from light and air (ie: a slide folder or slide box), designated as unfixed and be used within a 6 month period. |
| **Stock Solutions** | **Phosphate Buffer Solution, pH 7.0**  Purchased from Newcomer Supply **Wright’s-Giemsa Stain Solution**Purchased through Cardinal Health**Triton X, 10% Solution** Shelf life: 1 year at room temperatureTriton X (stock)..…………………………………….10.0 mL Distilled water …………………………………… …90.0 mL  |
| **Working Solutions** | The following solutions must be made fresh (Buffer-Stain Working Solution may be used up to four hours after preparation). Phosphate Buffer Solution, pH 7.0 Working Phosphate Buffer - Triton X Solution  Phosphate Buffer Solution, pH 7.0 ......................90.0 mL Triton X, 10% Working Solution (mix well)............10.0 mL Working Buffer- Stain Solution  Phosphate Buffer- Triton X working Solution………………..……...........45.0 mL (Save the remaining solution for rinsing; see Step 2 below). Stock Wright-Giemsa Stain……………………...........................…..….…5.0 mL |
| **Procedure** | • Stain 2 thin (routine) smears according to Wright/Giemsa Procedure.• Stain 1 thick prep slide and 1 Malaria control slide according to the Modified Giemsa Procedure-Malaria (below). DO NOT FIX PATIENT SLIDE. |
| **Step** | **Action** |
| 1 | Place the control slide and thick prep slide into the working Buffer-Stain Solution.………………. **50** minutes.  \*Keep the slides and solution covered during this step to prevent stain precipitation from forming on the smears. |
| 2 | Place slides directly into remaining working Buffer-Triton X Solution to remove excess stain……………………. **4** minutes **\*Do not overly agitate slides. Do not rinse slides in water after this step.** |
| 3 | Dry off slides. |
| 4 | Air-dry in a vertical position, and coverslip with synthetic mounting media. |
| 5 | Label slides with appropriate CoPath slide labels. Review Wright stain and Modified Giemsa stained slides and document on the QC Log for Hematology & Cytology Specimens. Return slides to Hematology for review. Slides should be then routed to assigned case pathologist. |
| **Procedure Notes** | This procedure was adapted from Detection of Parasites in Blood found in the Hematology manual. The control slides are available and purchased in a fixed state only. The patient thick prep is stained with Modified Giemsa unfixed; the red cells will lyse from the patient slide, but with little to no lysing of the control smear. (\*see “Note” under Quality Control section of this procedure). |
| **Results** | If positive, thick smear should have extracellular and/or intracellular RBC/WBC parasitic inclusions (blue). Control slide should demonstrate parasitic inclusions with minimal or no RBC lysis. |
| **Result Reporting** | By Pathologist |
| **References** | Hematology procedure manual: *Detection of Parasites in Blood.* 7/9/99 SOP HEM 8.5 MALP (Blood Parasites)Ash, L.R., et al, Parasites: A Guide to Laboratory Procedures and Identification, ASCP Press, Chicago, IL, 1987, pp 99-116 |

**Historical Record**

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| Version | Revised by | Effective Date | Summary of Revisions |
| 1 |  |  | Initial version. |
| 2 | A. Dubbelde | 6/27/19 | Update format, add version, and update to match current staining procedure used. |
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